Synthesis and Characterization of Luminescent Nano Hydroxyapatite (n-HAP)

A dissertation

Submitted for the partialfulfilmentof the

Degree of

Master of Science in Chemistry

Submitted by

Srikanta Moharana **&** **Susmita Mohanta**

Roll no: 410CY2019 **Roll no: 410CY2018**

Under the supervision of

Dr. Sasmita Mohapatra

Department of chemistry

National Institute of Technology (NIT)

Rourkela, Odisha, India.

Acknowledgement

We owe our cordial gratitude to my respected teacher and supervisor Dr. Sasmita Mohapatra, Assistant Professor, Department of Chemistry, National Institute of Technology, Rourkela, whose splendid guidance, authentic supervision, assiduous cooperation, moral support and constant encouragement enabled me to make out our research problem in the present form.

It is our great pleasure to acknowledge to Prof. B.G. Mishra, Head of the Chemistry Department, National Institute of Technology, Rourkela for providing us the necessary facilities for making this research work a success.

Weare highly indebted to all our teachers of this department for their kind help and expert suggestions. We express our profound gratitude to Mr. Smruti Ranjan Rout & Ms. Swagatika Sahu for their ceaseless encouragement, immense help and hearty encouragement during our project work.

We wish to thank all of friends for making our stay in this institute a memorable experience.

Finally, we must record our special attention to our parents & GOD who has always been a source of our strength, inspiration and our achievements.

Dr. Sasmita Mohapatra
Assistant Professor,
Department of Chemistry
National Institute of Technology
Rourkela
Odisha-769008

CERTIFICATE

This is to certify that the dissertation entitled, **"Synthesis and Characterization of Luminescent Nano Hydroxyapatite (n-HAP)"** submitted by Mr. Srikanta Moharana & Ms. Susmita Mohanta for the award of Master of Science in Chemistry during the period of August 2010- May2012 in the Department of Chemistry, National Institute of Technology, Rourkela, is a record of authentic work carried out by them under my supervision. To the best of my knowledge, the matter embodied in this dissertation has not been previously submitted for any degree in this/any other institute.

Date: **Dr. Sasmita Mohapatra**

Content

ABSTRACT

Luminescent mesoporous europium or terbium doped hydroxyapatite (Eu/Tb: HAp) was successfully prepared through a simple one-step co-precipitation method using block copolymer pluronic 123. The phase, morphology, composition and luminescent property of Eu/Tb: HAp powders were characterized by X-ray diffraction, scanning electron microscopy (SEM), dynamic light scattering (DLS), fluorescence spectrophotometry and energy dispersive X-ray spectroscopy (EDX). XRD analysis reveals the nanocrystalline nature of HAP with crystallite size 22 nm. SEM images indicate the formation of ultrafine particles with uniform spherical morphology. In Eu/Tb: HAP the maximum intensities were achieved at 588 and 544 nm respectively with corresponding excitations at 400 and 340 nm. The stability of Eu/Tb: HAp, investigated by measuring the hydrodynamic size against time shows that there is almost no change of hydrodynamic size even after several days. This observation implies that such stable nanosystems can be used as luminescent drug carriers which can circulate in the blood stream for a long period minimizing the rapid clearance of particles by macrophages

Keywords: Tb: Eu doped hydroxyapatite, Co-precipitation, Luminescence, Pluronic 123, Drug carrier1.

Introduction

Development of nano drug-carrier has great importance due to many advantages over conventional forms of dosage, such as enhanced bioavailability, greater efficiency, lower toxicity, controlled release, and so on. [1–7]Up to now, a large variety of nanosystems have been successfully employed as a means of sustained/controlled drug delivery, such as silica particles, [8] gold, [9]carbon nanotubes, [10]magnetic nanoparticles, [11] xerogels, [12] hydrogels.[13] However the major concerns for development of drug delivery Nano systems is their toxicity and biodegradability [14, 15]. Among different storage/release systems, calcium phosphate including hydroxyapatite (HAP) materials has gained enhanced interest with particular attention as drug storage and release hosts for biomedical applications due to their unique properties such as porosity, high specific surface area, non-toxicity, biocompatibility and well modifiability surface. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), the main mineral component of bones and teeth, is native to the human body. Combination of fluorescent properties onto HAP may enable the development of new multifunctional Nano composite for both therapeutic as well asbio imaging applications.

Most fluorescent materials used in general are organic dyes and/or CdSe/ZnS quantum dots (QDs). However the photo bleaching, quenching of dye molecules, toxicity of QDs limit their applications in biomedical field. Most recently, lanthanide doped inorganic nanomaterial's serve as a suitable alternative of these traditional fluorescent materials due to their excellent luminescence properties, including large Stokes shifts, narrow line–width emission bands, high quantum yields, long lifetimes and superior photo stability. As luminescent property of these materials arises from electronic transitions of these ions, their optical properties are rarely affected by surface modification.Trivalent rare earth ions doped hydroxyapatites have been found to exhibit favourable optical properties for use in laser hosts.[16-19]However to our knowledge, the preparation and application of luminescent HAP in drug delivery systems have been reported only rarely. The reason for this might be that HA has a complicated crystal structure (in comparison to common oxides, such as SiO_2) and it is very difficult to obtain stable porous HAP through traditional methods.It is well known that poly(ethylene glycol)–poly(propylene glycol)–poly(ethylene glycol) (PEG–PPG–PEG) block copolymer exhibits peculiar behaviours in aqueous solution. Below the characteristic temperature the critical micellar temperature (CMT), both the PEG and PPG blocks are soluble in water, and the copolymer molecules remain in the form of unimers in solution. At the CMT the PPG blocks become insoluble and form spherical micelles, with

PPO blocks as the hydrophobic core and the hydrated PEG blocks as the shell. The behaviours of block copolymer in water between the CMT and the cloud point (CP) have been extensively used to template various mesoporous materials [20-24]while its characteristics below CMT and at CP have often been used in cloud point extractions. [25, 26]Actually the behavioursbelow CMT and at CP of triblock copolymer–Pluronic P123 (EO 20 PO 70 EO 20), i.e. completely insoluble at CP and totally soluble below CMT, can be adjusted for the preparation of HAP.

The Tb^{3+}/Eu^{3+} doped HAP is one of the important green/red-emitting phosphors, and because the Tb^{3+}/Eu^{3+} ion is an important luminescent activator ion, showing emission due to $^5D_4 \rightarrow {}^7F_J$ transitions (J = 6, 5, 4, 3, 2)/$^5D_0 \rightarrow {}^7F_J$ transitions (J = 4, 3, 2, 1, 0) in the green/red region. [27–29] It is worth noting that porous materials functionalized with photoluminescence (PL) also have potential applications in the fields of drug delivery and disease diagnosis and therapy. [30–37] This is because these controlled drug delivery systems not only have high pore volumes for the storage and delivery of drugs but also possess photoluminescence properties which can be tracked to evaluate the efficiency of the drug release. [38, 39]

With this knowledge, we study the controlled synthesis of luminescent HAP using Tb^{3+}/Eu^{3+} as luminescent material. We used triblock copolymer pluronic P123 as template for the synthesis of porous Nano HAP (n-HAP). Results demonstrated that the effects of weight of copolymer have significant influence on hydrodynamic size and property of the obtained HAP. The synthesized porous luminescent HAP with high surface area can be used as a drug carrier and facilitates the controlled drug release of antibiotic cefradine.

2. Objective

Recently, much attention has been focused on fabrication of luminescent n-HAP for colloidal stability at physiological condition, high surface area for maximum drug loading, and biocompatibility. Therefore, synthesis of functionalized, highly water dispersible luminescent nanoparticles with high drug loading capacity by an easy method involving cheap and easily available starting materials is desirable. In this regard, our present investigation is addressed on the followings

- Synthesis of $Tb^{3+/}Eu^{3+-}$doped HAP luminescent nanoparticle using pluronic P-123 copolymer as template.

- Study the effect of copolymer weight used on hydrodynamic size and surface of n-HAP
- Characterization of the phase, surface, morphology, surface area overall hydrodynamic size and fluorescence properties using standard characterization techniques like X-ray diffraction technique, FTIR, Scanning electron microscope (SEM), BET, dynamic light scattering and fluorescence spectroscopy.
- Loading and release behaviours of model antibiotic cefradine

3. A Brief review of previous work

Owing to the challenging task for synthesis of luminescent porous HAP as drug carrier, this review focuses on various current synthetic strategies used to produce luminescent HAP and their physiological and optical properties. The knowledge of this review offers us valuable insight and inspiration to synthesize an efficient luminescent nano drug carrier based on biocompatible HAP.

Zhu et al reported a facile method a facile method for the fabrication of amorphous calcium phosphate (ACP) /polylactide-block-monomethoxy(polyethyleneglycol) hybrid nanoparticles and ACP porous nanospheres. [40] For luminescent property they performed Eu doping. Flurescence propery of the prepared sample was investigated and they found the most intense peak at 612 nm for 5 mol% Eu $^{3+}$ doping. Nano carrier is utilised for a slow and sustained ibuprofen drug release in simulated body fluid (SBF)

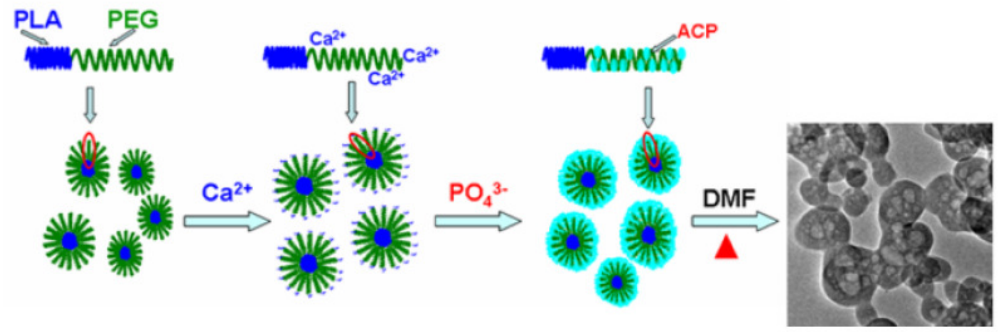


Fig.1. Strategy for the preparation of ACP/PLA-mPEG hybrid nanoparticles and ACP porous nanospheres [40]

HAP nano- and microcrystals with multiform morphologies (separated nanowires, nanorods, microspheres, microflowers, and microsheets) have been successfully synthesized

by Lin and co-worker using facile hydrothermal process.[41] They claimed that bright blue emission under long wavelength UV light is due to the presence of CO $2^{\bullet-}$ radical impurities in the crystal lattice. Furthermore, they found that organic additive (trisodium citrate) and pH values have an impact on the morphologies and luminescence properties of the products.

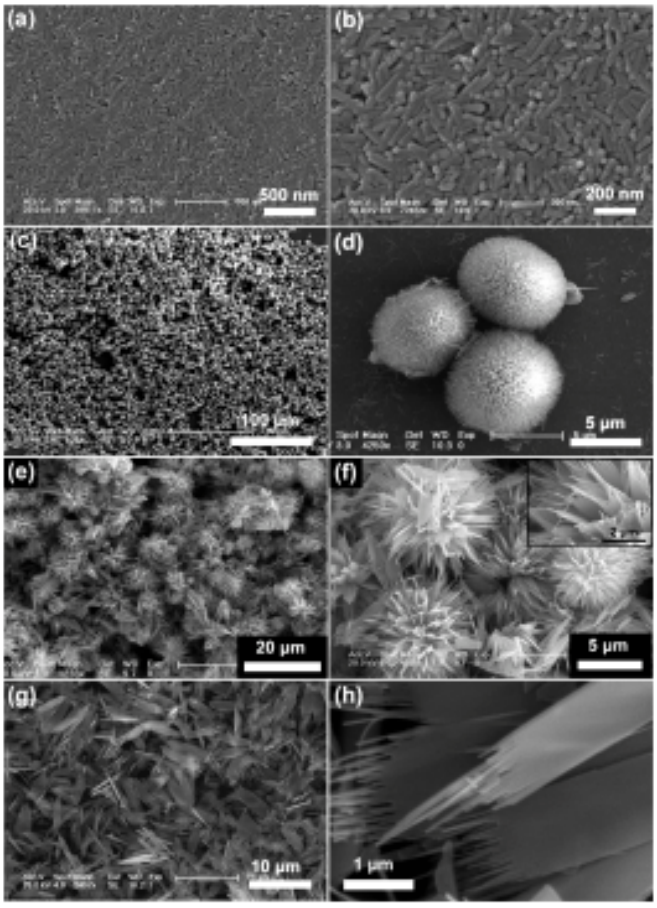


Fig.2. SEM images of HAP with different morphologies obtained at different pH values: (a, b) nanorods, pH) 7.0; (c, d) bur-like microspheres, pH) 5.0; (e, f) microflowers, pH) 4.5; and (g, h) microsheets, pH) 4.0.[41]

Tang et al reported a convenient strategy to obtain nano-HAP whose luminescence can be excited by visible lights. [42]They reported that in their method only the surface calcium ions on the HAP nanoparticles are partially replaced by a small amount of Tb^{3+}. It was also confirmed experimentally that the Tb-HAP nanoparticles can provide a steady luminescence The dimensions of the modified HAP are well controlled as their size distributions, 20 (5 nm, are relatively homogeneous.

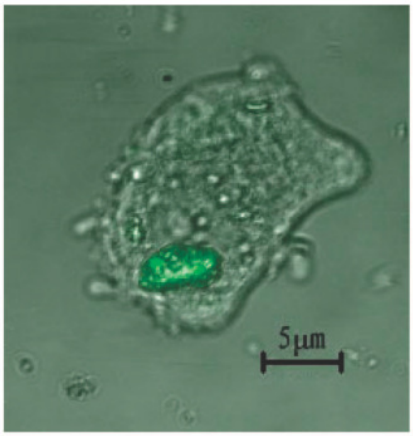


Fig.3. Green emission of the internalized Tb-HAP particles in the cells under confocal microscopy [42]

Bioactive, luminescent and mesoporous europium-doped hydroxyapatite (Eu:HAP) was successfully prepared by Lin et al. through a simple one-step route using cationic surfactant as template. [43] They used the system as a drug delivery carrier to investigate the drug storage/release properties using ibuprofen (IBU) as a model drug. The multifunctional HAP exhibits the typical mesoporous rod-like morphology with the particle size of 20–40 nm in width and 100–200 nm in length.

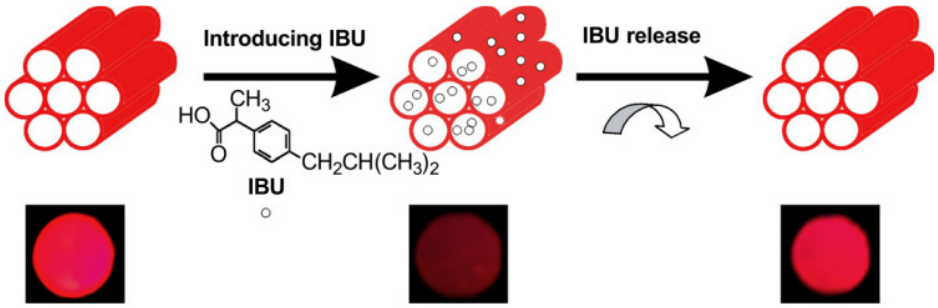


Fig.4. Schematic representation of loading and release of IBU on Eu:HAp with the corresponding pellet photographs under the irradiation of 365 nm UV lamp [43]

Ma and co-worker reported a citric acid sol-gel combustion method to synthesize terbium doped calcium phosphate (Tb-doped CaP) nanocrystalline powders.[44] The 4% Tb-doped CaP Nano crystalline powders exhibit the strongest emission at 548 nm (k excitation = 240 nm) and show strong green fluorescence under fluorescence microscopy.

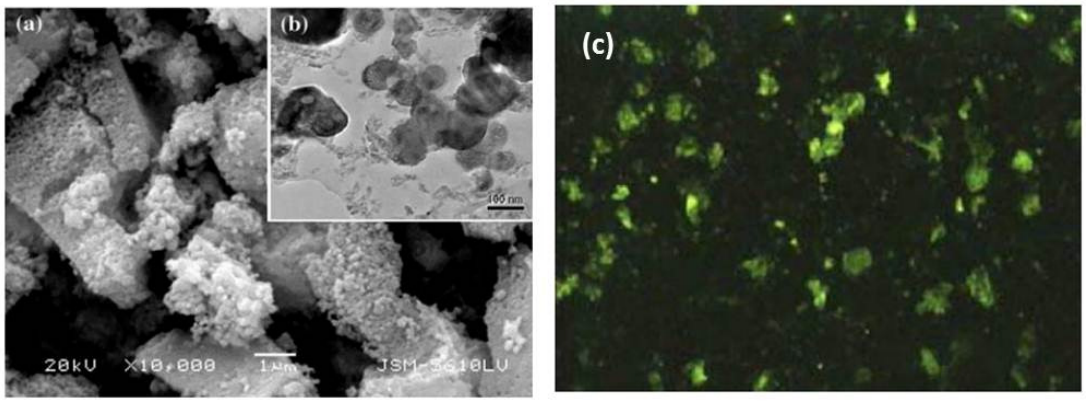


Fig.5. SEM (a), TEM (b) and Fluorescence microscopy (c) images of the 4% Tb-doped CaP nanocrystalline powders[44]

$Eu^{3+/}Gd^{3+}$-dual doped hydroxyapatite (HAP) nanorods were synthesized by Cui et al using hydrothermal method, they studied its photoluminescence (PL) properties, in vivo drug delivery and imaging.[45] The PL intensity of doped HAP nanorods can be adjusted by varying Eu^{3+} and Gd^{3+} concentrations. They found that doping of Eu^{3+} and Gd^{3+} have influence on the crystal growth of HAP, leading to smaller size and narrower size distribution of $Eu^{3+/}Gd^{3+}$-HAP nanorods compared with undoped HAP nanorods.

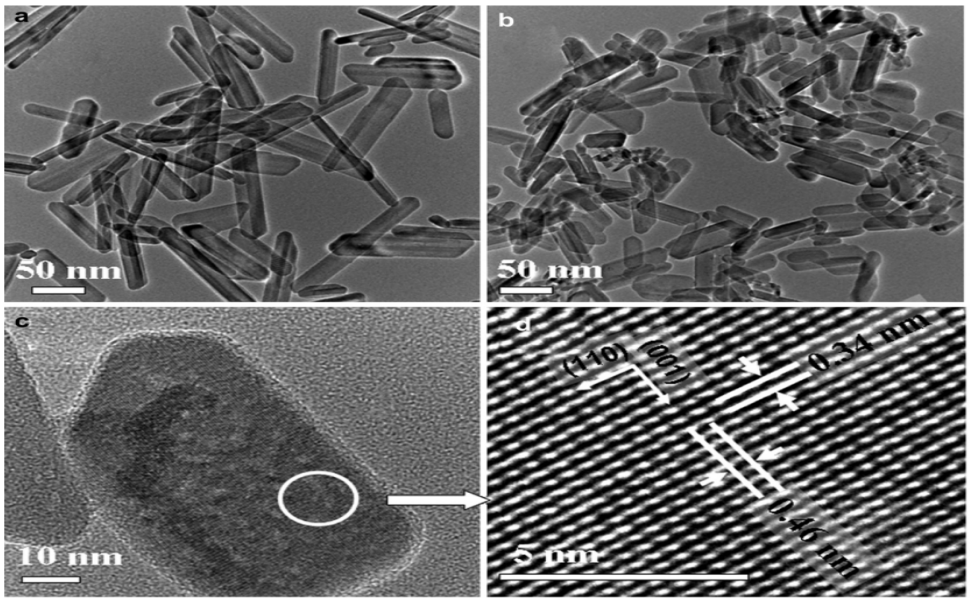


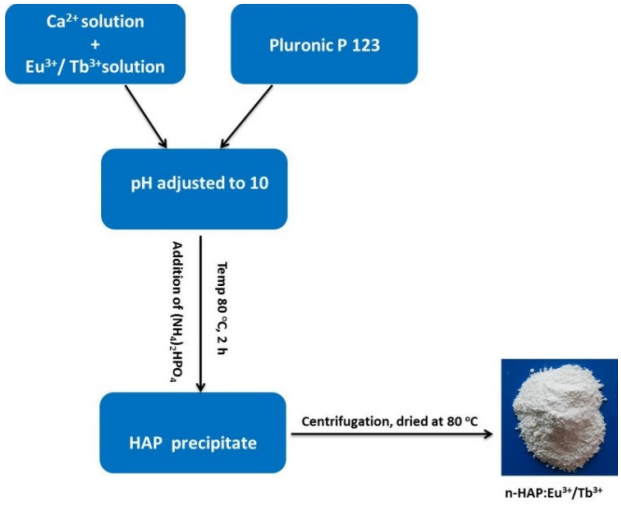
Fig. 6. TEM micrographs of the samples prepared by a microwave-assisted hydrothermal method at 200 ^{o}C for 30 min: (a) undoped HAp nanorods; (b-d) Eu^{3+}/Gd^{3+}-HAP nanorods (Eu^{3+} /Gd^{3+} molar ratio = 1:1)[45]

4. Characterization

The identification of crystalline phase of synthesized particles was performed by an Expert Pro Phillips X-ray diffractometer. The morphology and microstructure were analysed by scanning electron microscope (HITACHI COM-S-4200) operated at 300 kV. Mean particle diameters were measured after suitable dilution of the drug suspensions at 25.0 ± 0.5 oC, by laser light scattering using a particle size analyser (Nano ZS 90, Malvern). Measurements were performed at 90 ^{o} angle in 0.01 M phosphate buffer varying pH 2 to 11. FTIR spectra of as prepared, samples were obtained from a Thermo Nicolet Nexus FTIR model 870 spectrometer. Thermogravimetry analysis was used to analyse the weight percent of the organic components included in samples.

6. Results and Discussion

The synthesis of Tb/Eu doped n-HAP was carried out by simple co-precipitation method using copolymer pluronic P 123 as template (scheme-1).To investigate the amount of copolymer effect on the formation of the Nano crystalsize, the synthesis was done at various weight values ranging from 0.5 to 2 g. Pluronic P123 could control the size distribution of the precipitated n-HAP. There was no significant difference between the n-HAP particles with andwithout the Tb/Eu treatment.



Scheme-1: synthetic strategy for n-HAP-Eu^{3+}/Tb^{3+}

The composition and phase purity of the synthesised material is examined by XRD pattern. All the diffraction peaks of the synthesized nanoparticle can easily be indexed to pure hexagonal phase of HAP (JCPDS 09-0432). There is a large difference from each other in the

relative intensities of two samples in the XRD patterns (Fig. 7), indicating the possibility of perfect crystal growth on high temperature treatment. Moreover, as can be seen from the XRD patterns, high crystallinity can be realized at a relatively low treatment temperature (80 °C)

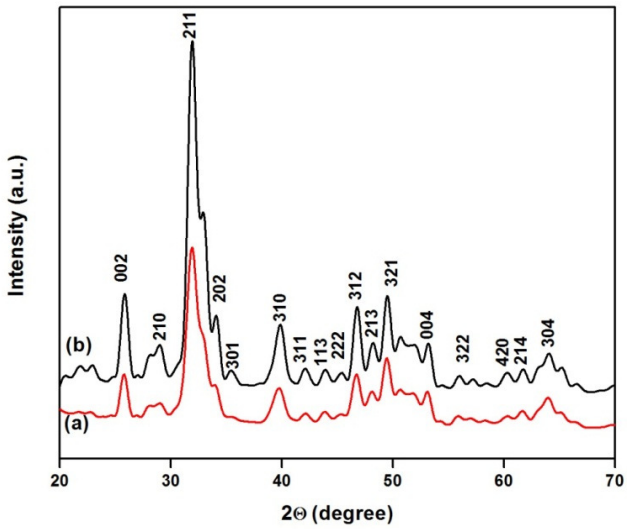


Fig.7. XRD pattern of n-HAP: Tb^{3+} (a) room temperature, (b) calcined sample

An investigation on hydrodynamic size of n-HAP:Tb^{3+} with respect to the copolymer amount used was carried out through dynamic light scattering (Fig. 9 a) in PBS. It is found that average hydrodynamic size of the nanoparticle is 42 nm. With increase in amount of copolymer from 0.5g to 1 g there is a significant decrease in hydrodynamic size from 40 nm to 25 nm, however with further increase in copolymer amount there is increase in hydrodynamic size appreciable first, then no significant change is observed. The stability of n-HAP: $Tb^{3+}$was investigated by measuring the hydrodynamic size against time (Fig. 9 b) shows that there is almost no change of hydrodynamic size even after several days. This observation implies that such stable nanosystems can be used as drug carrier which circulated in the blood stream for a long period minimizing the rapid clearance of particles by macrophages.

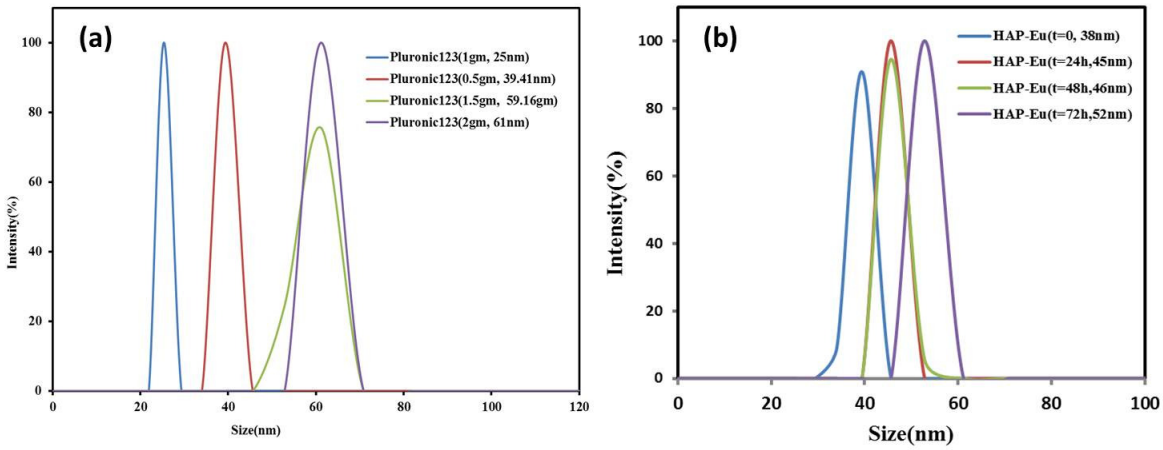


Fig.8. variation of hydrodynamic size with the amount of pluronic P123 (a) and time (b)

The FTIR spectrum of n-HAP:Tb^{3+}(Fig. 10) shows an intense broad splitted around 1100-1000 cm^{-1} due to stretching vibration of phosphate group. In addition to this presence of bands at 872,600,and 569 cm^{-1} due to bending vibration of phosphate strengthen the HAP formation. The presence of additional peaks at 1418, 1600cm^{-1}were due to CO_3^{2-},indicated that some carbonate group was incorporated into hydroxyapatite crystal structure replacing PO_4^{3-} groups.[46, 47]Appearance of peaks around 2800-2900 cm^{-1} indicates incomplete removal of copolymer during synthesis of n-HAP. The broad band around 3450 cm^{-1} is due to the hydroxyl stretching vibration of the combined water of n-HAP.

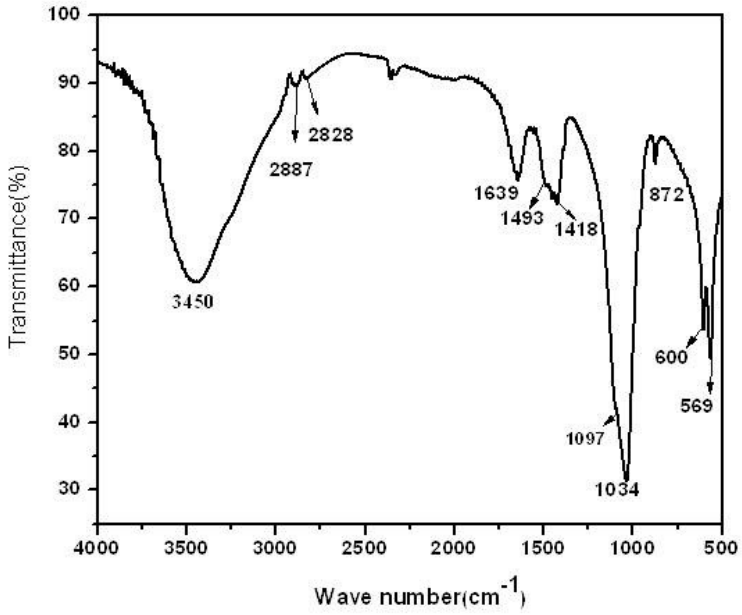


Fig.9. FTIR spectrum of n-HAP:Tb^{3+}

The surface properties of n-HAP:Tb^{3+} were further investigated by examining the surface charge over pH 2–10 by zeta potential measurement (Fig 11). It is observed that with increase in pH there is increase in zeta potential value.

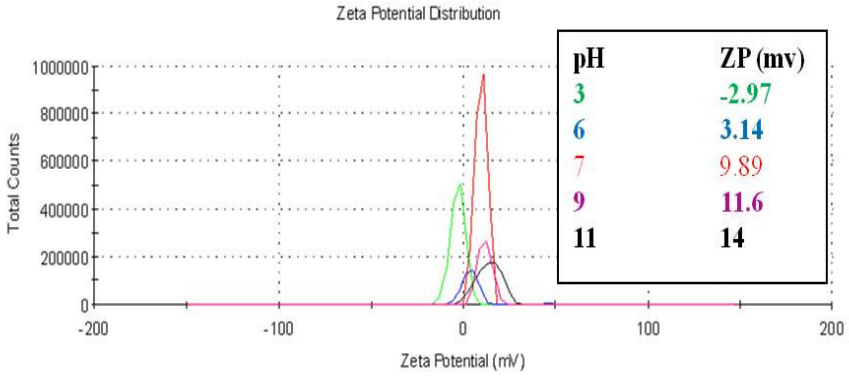


Fig.10. Variation of zeta potential of n-HAP with PH

In order to further investigate the surface chemistry, n-HAP:$Tb^{3+}$was studied using thermal analysis techniques of TG as shown in Fig.12. During heated to 800 ˚C, n-HAP showed three mass losses steps.The first mass loss occurs between 39 ^{o}C to 134 ^{o}C is due to the removal of moisture from surface of HAP nanoparticles. Continued heating resulted in subsequent slow (134 ^{o}C–500 ^{o}C) mass loss is due to decomposition of organic moiety present in pluronic P 123 along with lattice water. The gradual decrease in weight loss from 600 to 800^{o}C is due to the slow elimination of the carbonate group attached to the n-HAP nanoparticles. TG analysis shows that n-HAP is thermally stable up to 800 ^{o}C.

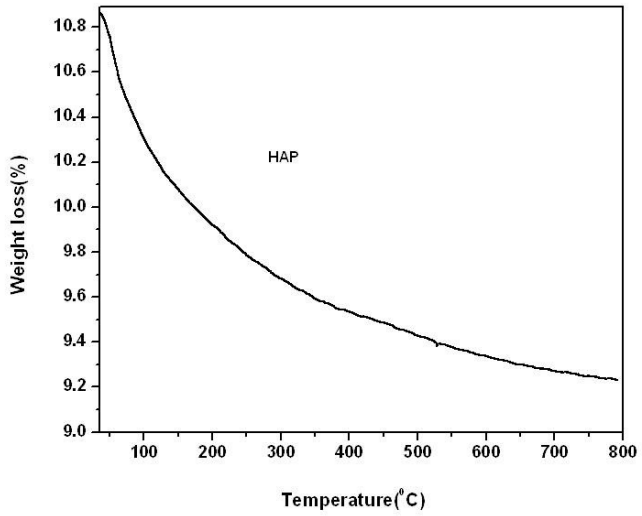


Fig.11. Thermo gravimetric curve of n-HAP:Tb^{3+}

7. Conclusion

We have successfully synthesised luminescent mesoporous n-HAP using a simple one-step co-precipitation method. This material exhibits high surface area, mesoporous, crystalline structure which can facilitate high drug encapsulation. As it shows high luminescent properties, it can be easily tracked and monitored in the drug release process. This system can be used for sustained and control release of antibiotic drug cefradine, which is under progress.

8. References

1. K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, K. M. Shakesheff, Chem. Rev., 1999, 99, 3181.
2. K. E. Fischer, B. J. Alem´an, S. L. Tao, R. H. Daniels, E. M. Li, M. D. Bunger., Nano Lett., 2009, 9, 716.
3. J. L. Vivero-Escoto, I. I. Slowing, C. W. Wu, V. S. Y. Lin, J. Am. Chem. Soc., 2009, 131, 3462.
4. W. Wei, G. H. Ma, G. Hu, D. Yu, T. Mcleish, Z. G. Su, J. Am. Chem .Soc., 2008, 130, 15808.
5. Q. Yang, S. C. Wang, P. W. Fan, L. F. Wang, Y. Di, K. F. Lin, Chem. Mater., 2005, 17, 5999.
6. W. Zhao, H. Chen, Y. Li, L. Li, M. Lang, J. Shi, Adv. Funct. Mater., 2008, 18, 2780.
7. A. Rosler, G. W. M. Vandermuelen, H. A. Klok, Adv. Drug Delivery Rev., 2001, 53, 95.
8. J. Choi, A. Burns, R.M. Williams, Z. Zhou, A. F. Nikitin, W.R. Zipfel, U. Wiesner, A.Y. Nikitin, J Biomed Opt., 2007, 12, 064007.
9. D. Kim, S. Park, J. H. Lee, Y.Y. Jeong, S. Jon, J .Am .Chem Soc., 2007, 129, 7661.
10. Z. Liu, W. Cai, L. He, N. Nakayama, K. Chen, X. Sun, X. Chen, H. Dai, Nanotech.,2007, 2, 47.
11. M. K. Yu, Y. Y. Jeong, J. Park, S. Park, J. W. Kim, J. J. Min, K. Kim, S. Jon, Angew Chem Int Ed., 2008, 47, 5362.
12. H. H. Yang, Q. Z. Zhu, H. Y. Qu, X. L. Chen, M. T. Din and J. G. Xu, Anal. Biochem., 2002, 71, 308.

13. M. Changez, K. Burugapalli, V. Koul and V. Choudhary, Biomaterials., 2003, 24, 527.
14. F . Tian, D. Cui, H. Schwarz, G.G. Estrada, H. Kobayashi, Toxicol In Vitro., 2006, 20, 1202.
15. I. Papageorgiou, C. Brown, R. Schins, S. Singh, R. Newson, S. Davis, J. Fisher, E.Ingham,C.P. Case, Biomaterials., 2007,28,2946.
16. L. D. Deloach, S. A. Payne, W. L. Kway , J. Lumin., 1994, 6, 85.
17. J.P.M. Van Vliet, G. Blasse, Mater Res Bull., 1990, 25, 391.
18. M. Tachihante, D. Zambon, A. Arbus , Mater Res Bull., 1993, 28, 605.
19. R. Ternane, M. T. Ayedi, N .Ariguib, J. Lumin., 1999, 81, 165.
20. D.Y. Zhao, Q. S Huo, J. L. Feng, B. F. Chmelka, G. D. Stucky, J. Am. Chem. Soc., 1998, 120, 6024.
21. J. M. Kim, G. D. Stucky, Chem. Com., 2000, 11, 59.
22. M. C. Burleigh, M. A. Markowitz, E. M. Wong, J. S. Lin, B. P. Gaber, Chem Mater., 2001,13,4411.
23. Y. F. Zhao, J. Ma, Micro. Meso. Mater., 2005, 87, 110.
24. L. Sierra, S. Valange, J. Barrault, J.L. Guth, Micro. Meso. Mater., 2008, 113, 352.
25. J. G. Huddleston, H. D. Willauer, S. T. Griffin, R. D. Rogers, Ind Eng Chem Res., 1999, 38, 2523.
26. H. Tani, Y. Suzuki , A. Matsuda , T. Kamidate, Anal Chim Acta., 2001, 429, 301.
27. P. Tanner, K.L. Wong, J. Phys. Chem. B., 2004, 108, 136.
28. E. M. Goldys, K. D. Tomsia, J. Sun, D. Dosev, I. M. Kennedy, S. Yatsunenko, M. Godlewski, J. Am. Chem. Soc., 2006, 128, 1449.
29. C. C. Chang, K. Fumiko, K. Tsunehisa, W. Hitoshi, Mater. Lett., 2005, 59, 1037.
30. D. L. Shi, J. Lian, W. Wang, G. K. Liu, P. He, Z. Y. Dong, L. M. Wang and R. C. Ewing, Adv. Mater., 2006, 18, 189.
31. Y. S. Lin, S. H. Wu, Y. Hung, Y. H. Chou, C. Chang, M. L. Lin, C. P. Tsai, C. Y. Mou, Chem. Mater., 2006, 18, 5170.
32. L. N. Sun, H. J. Zhang, C. Y. Peng, J. B. Yu, Q. G. Meng, J. Phys. Chem. B., 2006, 110, 7249.
33. L. M. Xiong, J. L. Shi, J. L. Gu, L. Li, W. M. Huang, J. H. Gao, M. L. Ruan, J. Phys. Chem. B, 2005, 109, 731.
34. C. Y. Peng, H. J. Zhang, J. B. Yu, Q. G. Meng, L. S. Fu, H. R. Li, J. Phys. Chem. B., 2005, 109, 15278.

35. J. Sauer, F. Marlow, B. Spliethoff, F. Schuth, Chem. Mater., 2002, 14, 217.
36. W. Xu, Y. T. Liao, D. L. Akins, J. Phys. Chem. B., 2002, 106, 11127.
37. C. M. Zhang, C. X. Li, S. S. Huang, Z. Y. Hou, Z. Y. Cheng, P. P. Yang, C. Peng and J. Lin, Biomaterials., 2010, 31, 3374.
38. Z. Y. Hou, P. P. Yang, H. Z. Lian, L. L. Wang, C. M. Zhang, C. X. Li, Z. Y. Hou, P. P. Yang, H. Z. Lian, L. L. Wang, C. M. Zhang, C. X. Li, R. T. Chai, Z. Y. Cheng, J. Lin, Chem. Eur. J., 2009, 15, 6973.
39. P. P. Yang, Z. W. Quan, C. X. Li, X. J. Kang, H. Z. Lian and J. Lin, Biomaterials., 2008, 29, 4341.
40. C. Feng , Y. J. Zhu, K. H. Zhang, J. Wu, K. W. Wang, Q. L. Tang, X. M. Mo, Nano Res Let., 2011, 6, 67.
41. C. Zhang, J. Yang, Z. Quan, P. Yang, C. Li, Z. Hou, J. Lin, Cryst. Growth & Design., 2009, 6, 2725.
42. L. Li, Y. Liu, J. Tao, M. Zhang, H. Pan, X. Xu, R. Tang, J. Phys. Chem. C., 2008, 112, 12219.
43. P. P. Yang, Z. W. Quan, C. X. Li, X. J. Kang, H. Z. Lian and J. Lin, Biomaterials., 2008, 29, 4341.
44. Y. Han, X. Wang, S. Li, X. Ma, J. Sol-Gel Sci. Technol., 2009, 49, 125.
45. C. Feng , H. Peng , Y.J. Zhu , J. Wu, C. L. Zhang, D. X. Cui, Biomaterials., 2011, 32, 9031.
46. M. Cao, Y. Wang, C. Guo, Y. Qi, Y. Hu, Langmuir., 2004, 20, 4784.
47. I. Manjubala, M. Sivakimar, Mater. Chem. Phys., 2001, 71, 272.